

1 INDIVIDUALIZED ANTI-CANCER ANTIBODIES

2 ~~Reference to Related Applications~~ REFERENCE TO RELATED

3 APPLICATIONS:

4 This application is a divisional of application S.N.  
5 09/727,361, filed November 29,2000, which is a continuation-  
6 in-part of application S.N. 09/415,278, filed October 8,  
7 1999, now U.S. Patent 6,180,357, the contents of  
8 which each are herein incorporated by reference.

9 ~~Field of the Invention~~ FIELD OF THE INVENTION:

10 This invention relates to the production of anti-cancer  
11 antibodies customized for the individual patient which may be  
12 combined with chemotherapeutic agents that can be used for  
13 therapeutic and diagnostic purposes. The invention further  
14 relates to the process by which the antibodies are made and  
15 to their methods of use.

16  
17 ~~Background of the Invention~~ BACKGROUND OF THE INVENTION:

18 Each individual who presents with cancer is unique and  
19 has a cancer that is as different from other cancers as that  
20 person's identity. Despite this, current therapy treats all  
21 patients with the same type of cancer, at the same stage, in  
22 the same way. At least 30% of these patients will fail the  
23 first line therapy, thus leading to further rounds of  
24 treatment and the increased probability of treatment failure,

1 metastases, and ultimately, death. A superior approach to  
2 treatment would be the customization of therapy for the  
3 particular individual. The only current therapy which lends  
4 itself to customization is surgery. Chemotherapy and  
5 radiation treatment can not be tailored to the patient, and  
6 surgery by itself, in most cases is inadequate for producing  
7 cures.

8 With the advent of monoclonal antibodies, the  
9 possibility of developing methods for customized therapy  
10 became more realistic since each antibody can be directed to  
11 a single epitope. Furthermore, it is possible to produce a  
12 combination of antibodies that are directed to the  
13 constellation of epitopes that uniquely define a particular  
14 individual's tumor.

15 Having recognized that a significant difference between  
16 cancerous and normal cells is that cancerous cells contain  
17 antigens that are specific to transformed cells, the  
18 scientific community has long held that monoclonal antibodies  
19 can be designed to specifically target transformed cells by  
20 binding specifically to these cancer antigens; thus giving  
21 rise to the belief that monoclonal antibodies can serve as  
22 "Magic Bullets" to eliminate cancer cells.

23 At the present time, however, the cancer patient usually  
24 has few options of treatment. The regimented approach to  
25 cancer therapy has produced improvements in global survival

1 and morbidity rates. However, to the particular individual,  
2 these improved statistics do not necessarily correlate with  
3 an improvement in their personal situation.

4 Thus, if a methodology was put forth which enabled the  
5 practitioner to treat each tumor independently of other  
6 patients in the same cohort, this would permit the unique  
7 approach of tailoring therapy to just that one person. Such  
8 a course of therapy would, ideally, increase the rate of  
9 cures, and produce better outcomes, thereby satisfying a  
10 long-felt need.

11 Historically, the use of polyclonal antibodies has been  
12 used with limited success in the treatment of human cancers.  
13 Lymphomas and leukemias have been treated with human plasma,  
14 but there were few prolonged remission or responses.  
15 Furthermore, there was a lack of reproducibility and there  
16 was no additional benefit compared to chemotherapy. Solid  
17 tumors such as breast cancers, melanomas and renal cell  
18 carcinomas have also been treated with human blood,  
19 chimpanzee serum, human plasma and horse serum with  
20 correspondingly unpredictable and ineffective results.

21 There have been many clinical trials of monoclonal  
22 antibodies for solid tumors. In the 1980s there were at least  
23 four clinical trials for human breast cancer which produced  
24 only one responder from at least 47 patients using antibodies  
25 against specific antigens or based on tissue selectivity. It

1 was not until 1998 that there was a successful clinical trial  
2 using a humanized anti-her 2 antibody in combination with  
3 Cisplatin. In this trial 37 patients were accessed for  
4 responses of which about a quarter had a partial response  
5 rate and another half had minor or stable disease  
6 progression.

7 The clinical trials investigating colorectal cancer  
8 involve antibodies against both glycoprotein and glycolipid  
9 targets. Antibodies such as 17-1A, which has some  
10 specificity for adenocarcinomas, had undergone Phase 2  
11 clinical trials in over 60 patients with only one patient  
12 having a partial response. In other trials, use of 17-1A  
13 produced only one complete response and two minor responses  
14 among 52 patients in protocols using additional  
15 cyclophosphamide. Other trials involving 17-1A yielded  
16 results that were similar. The use of a humanized murine  
17 monoclonal antibody initially approved for imaging also did  
18 not produce tumor regression. To date there has not been an  
19 antibody that has been effective for colorectal cancer.  
20 Likewise there have been equally poor results for lung  
21 cancer, brain cancers, ovarian cancers, pancreatic cancer,  
22 prostate cancer, and stomach cancer. There has been some  
23 limited success in the use of anti-GD3 monoclonal antibody  
24 for melanoma. Thus, it can be seen that despite successful  
25 small animal studies that are a prerequisite for human

1 clinical trials, the antibodies that have been tested have  
2 been for the most part ineffective.

3 ~~Prior Patents~~

4 PRIOR PATENTS:

5 U.S. Patent No. 5,750,102 discloses a process wherein  
6 cells from a patient's tumor are transfected with MHC genes  
7 which may be cloned from cells or tissue from the patient.  
8 These transfected cells are then used to vaccinate the  
9 patient.

10 U.S. Patent No. 4,861,581 discloses a process comprising  
11 the steps of obtaining monoclonal antibodies that are  
12 specific to an internal cellular component of neoplastic and  
13 normal cells of the mammal but not to external components,  
14 labeling the monoclonal antibody, contacting the labeled  
15 antibody with tissue of a mammal that has received therapy to  
16 kill neoplastic cells, and determining the effectiveness of  
17 therapy by measuring the binding of the labeled antibody to  
18 the internal cellular component of the degenerating  
19 neoplastic cells. In preparing antibodies directed to human  
20 intracellular antigens, the patentee recognizes that  
21 malignant cells represent a convenient source of such  
22 antigens.

23 U.S. Patent No. 5,171,665 provides a novel antibody and  
24 method for its production. Specifically, the patent teaches  
25 formation of a monoclonal antibody which has the property of

1 binding strongly to a protein antigen associated with human  
2 tumors, e.g. those of the colon and lung, while binding to  
3 normal cells to a much lesser degree.

4 U.S. Patent No. 5,484,596 provides a method of cancer  
5 therapy comprising surgically removing tumor tissue from a  
6 human cancer patient, treating the tumor tissue to obtain  
7 tumor cells, irradiating the tumor cells to be viable but  
8 non-tumorigenic, and using these cells to prepare a vaccine  
9 for the patient capable of inhibiting recurrence of the  
10 primary tumor while simultaneously inhibiting metastases.  
11 The patent teaches the development of monoclonal antibodies  
12 which are reactive with surface antigens of tumor cells. As  
13 set forth at col. 4, lines 45 et seq., the patentees utilize  
14 autochthonous tumor cells in the development of monoclonal  
15 antibodies expressing active specific immunotherapy in human  
16 neoplasia.

17 U.S. Patent No. 5,693,763 teaches a glycoprotein antigen  
18 characteristic of human carcinomas and not dependent upon the  
19 epithelial tissue of origin.

20 U.S. Patent No. 5,783,186 is drawn to Anti-Her2  
21 antibodies which induce apoptosis in Her2 expressing cells,  
22 hybridoma cell lines producing the antibodies, methods of  
23 treating cancer using the antibodies and pharmaceutical  
24 compositions including said antibodies.

1 U.S. Patent No. 5,849,876 describes new hybridoma cell  
2 lines for the production of monoclonal antibodies to mucin  
3 antigens purified from tumor and non-tumor tissue sources.

4 U.S. Patent No. 5,869,268 is drawn to a method for  
5 producing a human lymphocyte producing an antibody specific  
6 to a desired antigen, a method for producing a monoclonal  
7 antibody, as well as monoclonal antibodies produced by the  
8 method. The patent is particularly drawn to the production  
9 of an anti-HD human monoclonal antibody useful for the  
10 diagnosis and treatment of cancers.

11 U.S. Patent No. 5,869,045 relates to antibodies,  
12 antibody fragments, antibody conjugates and single chain  
13 immunotoxins reactive with human carcinoma cells. The  
14 mechanism by which these antibodies function is two-fold, in  
15 that the molecules are reactive with cell membrane antigens  
16 present on the surface of human carcinomas, and further in  
17 that the antibodies have the ability to internalize within  
18 the carcinoma cells, subsequent to binding, making them  
19 especially useful for forming antibody-drug and antibody-  
20 toxin conjugates. In their unmodified form the antibodies  
21 also manifest cytotoxic properties at specific  
22 concentrations.

23 U.S. Patent No. 5,780,033 discloses the use of  
24 autoantibodies for tumor therapy and prophylaxis. However,  
25 this antibody is an antinuclear autoantibody from an aged

1 mammal. In this case, the autoantibody is said to be one type  
2 of natural antibody found in the immune system. Because the  
3 autoantibody comes from "an aged mammal", there is no  
4 requirement that the autoantibody actually comes from the  
5 patient being treated. In addition the patent discloses  
6 natural and monoclonal antinuclear autoantibody from an aged  
7 mammal, and a hybridoma cell line producing a monoclonal  
8 antinuclear autoantibody.

9  
10 ~~Summary of the Invention~~ SUMMARY OF THE INVENTION:

11 This application teaches a method for producing patient  
12 specific anti-cancer antibodies using a novel paradigm of  
13 screening. These antibodies can be made specifically for one  
14 tumor and thus make possible the customization of cancer  
15 therapy. Within the context of this application, anti-cancer  
16 antibodies having either cell-killing (cytotoxic) or cell-  
17 growth inhibiting (cytostatic) properties will hereafter be  
18 referred to as cytotoxic. These antibodies can be used in  
19 aid of staging and diagnosis of a cancer, and can be used to  
20 treat tumor metastases.

21 The prospect of individualized anti-cancer treatment  
22 will bring about a change in the way a patient is managed. A  
23 likely clinical scenario is that a tumor sample is obtained  
24 at the time of presentation, and banked. From this sample,  
25 the tumor can be typed from a panel of pre-existing anti-



1 cancer antibodies. The patient will be conventionally staged  
2 but the available antibodies can be of use in further staging  
3 the patient. The patient can be treated immediately with the  
4 existing antibodies, and a panel of antibodies specific to  
5 the tumor can be produced either using the methods outlined  
6 herein or through the use of phage display libraries in  
7 conjunction with the screening methods herein disclosed. All  
8 the antibodies generated will be added to the library of  
9 anti-cancer antibodies since there is a possibility that  
10 other tumors can bear some of the same epitopes as the one  
11 that is being treated.

12 In addition to anti-cancer antibodies, the patient can  
13 elect to receive the currently recommended therapies as part  
14 of a multi-modal regimen of treatment. The fact that the  
15 antibodies isolated via the present methodology are  
16 relatively non-toxic to non-cancerous cells allow  
17 combinations of antibodies at high doses to be used, either  
18 alone, or in conjunction with conventional therapy. The high  
19 therapeutic index will also permit re-treatment on a short  
20 time scale that should decrease the likelihood of emergence  
21 of treatment resistant cells.

22 If the patient is refractory to the initial course of  
23 therapy or metastases develop, the process of generating  
24 specific antibodies to the tumor can be repeated for re-  
25 treatment. Furthermore, the anti-cancer antibodies can be

1 conjugated to red blood cells obtained from that patient and  
2 re-infused for treatment of metastases. There have been few  
3 effective treatments for metastatic cancer and metastases  
4 usually portend a poor outcome resulting in death. However,  
5 metastatic cancers are usually well vascularized and the  
6 delivery of anti-cancer antibodies by red blood cells can  
7 have the effect of concentrating the antibodies at the site  
8 of the tumor. Even prior to metastases, most cancer cells  
9 are dependent on the host's blood supply for their survival  
10 and anti-cancer antibody conjugated red blood cells can be  
11 effective against *in situ* tumors, too. Alternatively, the  
12 antibodies may be conjugated to other hematogenous cells,  
13 e.g. lymphocytes, macrophages, monocytes, natural killer  
14 cells, etc.

15       There are five classes of antibodies and each is  
16 associated with a function that is conferred by its heavy  
17 chain. It is generally thought that cancer cell killing by  
18 naked antibodies are mediated either through antibody  
19 dependent cellular cytotoxicity or complement dependent  
20 cytotoxicity. For example murine IgM and IgG2a antibodies  
21 can activate human complement by binding the C-1 component of  
22 the complement system thereby activating the classical  
23 pathway of complement activation which can lead to tumor  
24 lysis. For human antibodies the most effective complement  
25 activating antibodies are generally IgM and IgG1. Murine

1 antibodies of the IgG2a and IgG3 isotype are effective at  
2 recruiting cytotoxic cells that have Fc receptors which will  
3 lead to cell killing by monocytes, macrophages, granulocytes  
4 and certain lymphocytes. Human antibodies of both the IgG1  
5 and IgG3 isotype mediate ADCC.

6 Another possible mechanism of antibody mediated cancer  
7 killing may be through the use of antibodies that function to  
8 catalyze the hydrolysis of various chemical bonds in the cell  
9 membrane and its associated glycoproteins or glycolipids, so-  
10 called catalytic antibodies.

11 There are two additional mechanisms of antibody mediated  
12 cancer cell killing which are more widely accepted. The  
13 first is the use of antibodies as a vaccine to induce the  
14 body to produce an immune response against the putative  
15 cancer antigen that resides on the tumor cell. The second is  
16 the use of antibodies to target growth receptors and  
17 interfere with their function or to down regulate that  
18 receptor so that effectively its function is lost.

19 Accordingly, it is an objective of the invention to  
20 teach a method for producing anti-cancer antibodies from  
21 cells derived from a particular individual which are  
22 cytotoxic with respect to cancer cells while simultaneously  
23 being relatively non-toxic to non-cancerous cells.

24 It is an additional objective of the invention to  
25 produce novel anti-cancer antibodies.

1           It is a further objective of the instant invention to  
2   produce anti-cancer antibodies whose cytotoxicity is mediated  
3   through antibody dependent cellular toxicity.

4           It is yet an additional objective of the instant  
5   invention to produce anti-cancer antibodies whose  
6   cytotoxicity is mediated through complement dependent  
7   cellular toxicity.

8           It is still a further objective of the instant invention  
9   to produce anti-cancer antibodies whose cytotoxicity is a  
10   function of their ability to catalyze hydrolysis of cellular  
11   chemical bonds.

12          Still an additional objective of the instant invention  
13   is to produce anti-cancer antibodies useful as a vaccine to  
14   produce an immune response against putative cancer antigen  
15   residing on tumor cells.

16          A further objective of the instant invention is the use  
17   of antibodies to target cell membrane proteins, such as  
18   growth receptors, cell membrane pumps and cell anchoring  
19   proteins, thereby interfering with or down regulating their  
20   function.

21          Yet an additional objective of the instant invention is  
22   the production of anti-cancer antibodies whose cell-killing  
23   utility is concomitant with their ability to effect a  
24   conformational change in cellular proteins such that a signal  
25   will be transduced to initiate cell-killing.

1           A still further objective of the instant invention is to  
2     produce anti-cancer antibodies which are useful for  
3     diagnosis, prognosis, and monitoring of cancer, e.g.  
4     production of a panel of therapeutic anti-cancer antibodies  
5     to test patient samples to determine if they contain any  
6     suitable antibodies for therapeutic use.

7           Yet another objective of the instant invention is to  
8     produce novel antigens, associated with cancer processes,  
9     which can be discovered by using anti-cancer antibodies  
10    derived by the process of the instant invention. These  
11    antigens are not limited to proteins, as is generally the  
12    case with genomic data; they may also be derived from  
13    carbohydrates or lipids or combinations thereof.

14          Other objects and advantages of this invention will  
15    become apparent from the following description wherein are  
16    set forth, by way of illustration and example, certain  
17    embodiments of this invention.

18  
19    ~~Detailed Description of the Invention~~ DETAILED DESCRIPTION OF  
20    THE INVENTION:

21          It is to be understood that while a certain form of the  
22    invention is illustrated, it is not to be limited to the  
23    specific form or arrangement herein described and shown. It  
24    will be apparent to those skilled in the art that various  
25    changes may be made without departing from the scope of the

1 invention and the invention is not to be considered limited  
2 to what is shown and described in the specification.

3 One of the potential benefits of monoclonal antibodies  
4 with respect to the treatment of cancer is their ability to  
5 specifically recognize single antigens. It was thought that  
6 in some instances cancer cells possess antigens that were  
7 specific to that kind of transformed cell. It is now more  
8 frequently believed that cancer cells have few unique  
9 antigens, rather, they tend to over-express a normal antigen  
10 or express fetal antigens. Nevertheless, the use of  
11 monoclonal antibodies provided a method of delivering  
12 reproducible doses of antibodies to the patient with the  
13 expectation of better response rates than with polyclonal  
14 antibodies.

15 Traditionally, monoclonal antibodies have been made  
16 according to fundamental principles laid down by Kohler and  
17 Milstein. Mice are immunized with antigens, with or without,  
18 adjuvants. The splenocytes are harvested from the spleen for  
19 fusion with immortalized hybridoma partners. These are  
20 seeded into microtitre plates where they can secrete  
21 antibodies into the supernatant that is used for cell  
22 culture. To select from the hybridomas that have been plated  
23 for the ones that produce antibodies of interest the  
24 hybridoma supernatants are usually tested for antibody  
25 binding to antigens in an ELISA (enzyme linked immunosorbent

1 assay) assay. The idea is that the wells that contain the  
2 hybridoma of interest will contain antibodies that will bind  
3 most avidly to the test antigen, usually the immunizing  
4 antigen. These wells are then subcloned in limiting dilution  
5 fashion to produce monoclonal hybridomas. The selection for  
6 the clones of interest is repeated using an ELISA assay to  
7 test for antibody binding. Therefore, the principle that has  
8 been propagated is that in the production of monoclonal  
9 antibodies the hybridomas that produce the most avidly  
10 binding antibodies are the ones that are selected from among  
11 all the hybridomas that were initially produced. That is to  
12 say, the preferred antibody is the one with highest affinity  
13 for the antigen of interest.

14 There have been many modifications of this procedure  
15 such as using whole cells for immunization. In this method,  
16 instead of using purified antigens, entire cells are used for  
17 immunization. Another modification is the use of cellular  
18 ELISA for screening. In this method instead of using  
19 purified antigens as the target in the ELISA, fixed cells are  
20 used. In addition to ELISA tests, complement mediated  
21 cytotoxicity assays have also been used in the screening  
22 process. However, antibody-binding assays were used in  
23 conjunction with cytotoxicity tests. Thus, despite many  
24 modifications, the process of producing monoclonal antibodies

1 relies on antibody binding to the test antigen as an  
2 endpoint.

3 Most antibodies directed against cancer cells have been  
4 produced using the traditional methods outlined above. These  
5 antibodies have been used both therapeutically and  
6 diagnostically. In general, for both these applications, the  
7 antibody has been used as the targeting agent that delivers a  
8 payload to the site of the cancer. These antibody conjugates  
9 can either be radioactive, toxic, or serve as an intermediary  
10 for further delivery of a drug to the body, such as an enzyme  
11 or biotin. Furthermore, it was widely held, until recently,  
12 that naked antibodies had little effect *in vivo*. Both  
13 HERCEPTIN and RITUXIMAB are humanized murine monoclonal  
14 antibodies that have recently been approved for human use by  
15 the FDA. However, both these antibodies were initially made  
16 by assaying for antibody binding and their direct  
17 cytotoxicity was not the primary goal during the production  
18 of hybridomas. Any tendency for these antibodies to produce  
19 tumor cell killing is thus through chance, not by design.

20 Although the production of monoclonal antibodies have  
21 been carried out using whole cell immunization for various  
22 applications the screening of these hybridomas have relied on  
23 either putative or identified target antigens or on the  
24 selectivity of these hybridomas for specific tissues. It is  
25 axiomatic that the best antibodies are the ones with the



1 highest binding constants. This concept originated from the  
2 basic biochemical principle that enzymes with the highest  
3 binding constants were the ones that were the most effective  
4 for catalyzing a reaction. This concept is applicable to  
5 receptor ligand binding where the drug molecule binding to  
6 the receptor with the greatest affinity usually has the  
7 highest probability for initiating or inhibiting a signal.  
8 However, this may not always be the case since it is possible  
9 that in certain situations there may be cases where the  
10 initiation or inhibition of a signal may be mediated through  
11 non-receptor binding. The information conveyed by a  
12 conformational change induced by ligand binding can have many  
13 consequences such as a signal transduction, endocytosis,  
14 among the others. The ability to produce a conformational  
15 change in a receptor molecule may not necessarily be due to  
16 the filling of a ligand receptor pocket but may occur through  
17 the binding of another extra cellular domain or due to  
18 receptor clustering induced by a multivalent ligand.

19 The production of antibodies to produce cell killing  
20 need not be predicated upon screening of the hybridomas for  
21 the best binding antibodies. Rather, although not advocated  
22 by those who produce monoclonal antibodies, the screening of  
23 the hybridoma supernatants for cell killing or alternatively  
24 for cessation of growth of the cancerous cells may be  
25 selected as a desirable endpoint for the production of

1. cytotoxic or cytostatic antibodies. It is well understood  
2 that the *in-vivo* antibodies mediate their function through  
3 the Fc portions and that the utility of the therapeutic  
4 antibody is determined by the functionality of the constant  
5 region or attached moieties. In this case the FAb portion of  
6 the antibody, the antigen-combining portion, will confer to  
7 the antibody its specificity and the Fc portion its  
8 functionality. The antigen combining site of the antibody  
9 can be considered to be the product of a natural  
10 combinatorial library. The result of the rearrangement of the  
11 variable region of the antibody can be considered a molecular  
12 combinatorial library where the output is a peptide.  
13 Therefore, the sampling of this combinatorial library can be  
14 based on any parameter. Like sampling a natural compound  
15 library for antibiotics, it is possible to sample an antibody  
16 library for cytotoxic or cytostatic compounds.

17       The various endpoints in a screen must be differentiated  
18 from each other. For example, the difference between antibody  
19 binding to the cell is distinct from cell killing. Cell  
20 killing (cytotoxicity) is distinct from the mechanisms of  
21 cell death such as oncosis or apoptosis. There can be many  
22 processes by which cell death is achieved and some of these  
23 can lead either to oncosis or apoptosis. There is speculation  
24 that there are other cell death mechanisms other than oncosis  
25 or apoptosis but regardless of how the cell arrives at death

1 there are some commonalities of cell death. One of these is  
2 the absence of metabolism and another is the denaturation of  
3 enzymes. In either case vital stains will fail to stain these  
4 cells. These endpoints of cell death have been long  
5 understood and predate the current understanding of the  
6 mechanisms of cell death. Furthermore, there is the  
7 distinction between cytotoxic effects where cells are killed  
8 and cytostatic effects where the proliferation of cells are  
9 inhibited.

10 In a preferred embodiment of the present invention, the  
11 assay is conducted by focusing on cytotoxic activity toward  
12 cancerous cells as an end point. In a preferred embodiment,  
13 a live /dead assay kit , for example the LIVE/DEAD<sup>®</sup>  
14 Viability/Cytotoxicity Assay Kit (L-3224) by Molecular  
15 Probes, is utilized. The Molecular Probes kit provides a  
16 two-color fluorescence cell viability assay that is based on  
17 the simultaneous determination of live and dead cells with  
18 two probes that measure two recognized parameters of cell  
19 viability - intracellular esterase activity and plasma  
20 membrane integrity. The assay principles are general and  
21 applicable to most eukaryotic cell types, including adherent  
22 cells and certain tissues, but not to bacteria or yeast.  
23 This fluorescence-based method of assessing cell viability is  
24 preferred in place of such assays as trypan blue exclusion,

1 Cr release and similar methods for determining cell viability  
2 and cytotoxicity.

3 In carrying out the assay, live cells are distinguished  
4 by the presence of ubiquitous intracellular esterase  
5 activity, determined by the enzymatic conversion of the  
6 virtually nonfluorescent cell-permeant CALCEIN AM to the  
7 intensely fluorescent Calcein. The polyanionic dye Calcein  
8 is well retained within live cells, producing an intense  
9 uniform green fluorescence in live cells (ex/em ~495 nm/~515  
10 nm). EthD-1 enters cells with damaged membranes and  
11 undergoes a 40-fold enhancement of fluorescence upon binding  
12 to nucleic acids, thereby producing a bright red fluorescence  
13 in dead cells (ex/em ~495 nm/~635 nm). EthD-1 is excluded by  
14 the intact plasma membrane of live cells. The determination  
15 of cell viability depends on these physical and biochemical  
16 properties of cells. Cytotoxic events that do not affect  
17 these cell properties may not be accurately assessed using  
18 this method. Background fluorescence levels are inherently  
19 low with this assay technique because the dyes are virtually  
20 nonfluorescent before interacting with cells.

21 In addition to the various endpoints for screening,  
22 there are two other major characteristics of the screening  
23 process. The library of antibody gene products is not a  
24 random library but is the product of a biasing procedure. In  
25 the examples below, the biasing is produced by immunizing

1 mice with fixed cells. This increases the proportion of  
2 antibodies that have the potential to bind the target  
3 antigen. Although immunization is thought of as a way to  
4 produce higher affinity antibodies (affinity maturation) in  
5 this case it is not. Rather, it can be considered as a way  
6 to shift the set of antigen combining sites towards the  
7 targets. This is also distinct from the concept of isotype  
8 switching where the functionality, as dictated by the  
9 constant portion of the heavy chain, is altered from the  
10 initial IgM isotype to another isotype such as IgG.

11       The third key feature that is crucial in the screening  
12 process is the use of multitarget screening. To a certain  
13 extent specificity is related to affinity. An example of this  
14 is the situation where an antigen has very limited tissue  
15 distribution and the affinity of the antibody is a key  
16 determinant of the specificity of the antibody-the higher the  
17 affinity the more tissue specific the antibody and likewise  
18 an antibody with low affinity may bind to tissues other than  
19 the one of interest. Therefore, to address the specificity  
20 issue the antibodies are screened simultaneously against a  
21 variety of cells. In the examples below the hybridoma  
22 supernatants (representing the earliest stages of monoclonal  
23 antibody development), are tested against a number of cell  
24 lines to establish specificity as well as activity.

1       The antibodies are designed for therapeutic treatment of  
2 cancer in patients. Ideally the antibodies can be naked  
3 antibodies. They can also be conjugated to toxins. They can  
4 be used to target other molecules to the cancer. e.g. biotin  
5 conjugated enzymes. Radioactive compounds can also be used  
6 for conjugation.

7       The antibodies can be fragmented and rearranged  
8 molecularly. For example Fv fragments can be made; sFv-single  
9 chain Fv fragments; diabodies etc.

10       It is envisioned that these antibodies can be used for  
11 diagnosis, prognosis, and monitoring of cancer. For example  
12 the patients can have blood samples drawn for shed tumor  
13 antigens which can be detected by these antibodies in  
14 different formats such as ELISA assays, rapid test panel  
15 formats etc. The antibodies can be used to stain tumor  
16 biopsies for the purposes of diagnosis. In addition a panel  
17 of therapeutic antibodies can be used to test patient samples  
18 to determine if there are any suitable antibodies for  
19 therapeutic use.

20       ~~Example one~~   The hybridoma cell lines 1LN-8 (shown in the  
21 table on page 31), 3BD-8 (shown in the table on page 27),  
22 3BD-26 (shown in the table on page 27), 3BD-27 (shown in the  
23 table on page 27), H460-27 (shown in the table on page 46),  
24 H460-23 (shown in the table on page 46), 7BD-14 (shown in the

1 table on page 36) and 5LAC20 (shown in the table on page 42)  
2 were deposited, in accordance with the Budapest Treaty, with  
3 the American Type Culture Collection (ATCC), 10801 University  
4 Blvd., Manassas, VA 20110-2209 on November 21, 2000 under  
5 Accession Numbers PTA-2693, PTA-2696, PTA-2695, PTA-2698,  
6 PTA-2699, PTA-2700, PTA-2697 and PTA-2694 respectively. The  
7 hybridoma cell lines H460-16-2 (shown in the table on page  
8 46) and 7BDI-60 (shown in the table on page 36) were deposited  
9 on September 4, 2002 under Accession Numbers PTA-4621 and  
10 PTA-4623 respectively. In accordance with 37 CFR 1.808, the  
11 depositors assure that all restrictions imposed on the  
12 availability to the public of the deposited materials will be  
13 irrevocably removed upon the granting of a patent. The  
14 depositors additionally assure that the deposited materials  
15 will be replaced if viable samples cannot be dispensed by the  
16 depository.

#### 17 EXAMPLE ONE

18 In order to produce monoclonal antibodies specific for a  
19 tumor sample the method of selection of the appropriate  
20 hybridoma wells is complicated by the probability of  
21 selecting wells which will produce false positive signals.  
22 That is to say that there is the likelihood of producing  
23 antibodies that can react against normal cells as well as  
24 cancer cells. To obviate this possibility one strategy is to  
25 mask the anti-normal antigen antibodies from the selection

1 process. This can be accomplished by removing the anti-  
2 normal antibodies at the first stage of screening thereby  
3 revealing the presence of the desired antibodies. Subsequent  
4 limiting dilution cloning can delineate the clones that will  
5 not produce killing of control cells but will produce target  
6 cancer cell killing.

7 Biopsy specimens of breast, melanoma, and lung tumors  
8 were obtained and stored at  $-70^{\circ}\text{C}$  until used. Single cell  
9 suspensions were prepared and fixed with  $-30^{\circ}\text{C}$ , 70% ethanol,  
10 washed with PBS and reconstituted to an appropriate volume  
11 for injection. Balb/c mice were immunized with  $2.5 \times 10^5$ - $1 \times 10^6$   
12 cells and boosted every third week until a final pre-fusion  
13 boost was performed three days prior to the splenectomy. The  
14 hybridomas were prepared by fusing the isolated splenocytes  
15 with Sp2/0 and NS1 myeloma partners. The supernatants from  
16 the fusions were tested for subcloning of the hybridomas.

17 Cells (including A2058 melanoma cells, CCD-12CoN fibroblasts,  
18 MCF-12A breast cells among others) were obtained from ATCC  
19 and cultured according to enclosed instructions. The HEY cell  
20 line was a gift from Dr. Inka Brockhausen. The non-cancer  
21 cells, e.g. CCD-12CoN fibroblasts and MCF-12A breast cells,  
22 were plated into 96-well microtitre plates (NUNC) 1 to 2  
23 weeks prior to screening. The cancer cells, e.g. HEY, A2058,  
24 BT 483, and HS294t, were plated two or three days prior to  
25 screening.



1       The plated normal cells were fixed prior to use. The  
2 plates were washed with 100 microliters of PBS for 10 minutes  
3 at room temperature and then aspirated dry. 75 microliters  
4 of 0.01 percent glutaraldehyde diluted in PBS were added to  
5 each well for five minutes and then aspirated. The plates  
6 were washed with 100 microliters of PBS three times at room  
7 temperature. The wells were emptied and 100 microliters of  
8 one percent human serum albumin in PBS was added to each well  
9 for one hour at room temperature. The plates were then  
10 stored at four degrees Celsius.

11       Prior to the transfer of the supernatant from the  
12 hybridoma plates the fixed normal cells were washed three  
13 times with 100 microliters of PBS at room temperature. After  
14 aspiration to the microliters of the primary hybridoma  
15 culture supernatants were transferred to the fixed cell  
16 plates and incubated for two hours at 37 degrees Celsius in a  
17 8 percent CO<sub>2</sub> incubator. The hybridoma supernatants derived  
18 from melanoma was incubated with CCD-12 CoN cells and those  
19 derived from breast cancer were incubated with MCF-12a cells.

20       After incubation the absorbed supernatant was divided  
21 into two 75 microliter portions and transferred to target  
22 cancer cell plates. Prior to the transfer the cancer cell  
23 plates were washed three times with 100 microliters of PBS.  
24 The supernatant from the CCD-12 CoN cells were transferred to  
25 the A2058 and the HS294t cells, whereas the supernatant from

1 MCF-12A cells were transferred to the HEY and BT 483 cells.  
2 The cancer cells were incubated with the hybridoma  
3 supernatants for 18 hours at 37 degrees Celsius in an 8  
4 percent CO<sub>2</sub> incubator.

5 The ~~Live/Dead~~LIVE/DEAD cytotoxicity assay was obtained  
6 from Molecular Probes (Eu,OR). The assays were performed  
7 according to the manufacturer's instructions with the changes  
8 outlined below. The plates with the cells were washed once  
9 with 100 microliters of PBS at 37°C. 75 to 100 microliters  
10 of supernatant from the hybridoma microtitre plates were  
11 transferred to the cell plates and incubated in a 8% CO<sub>2</sub>  
12 incubator for 18-24 hours. Then, the wells that served as the  
13 all dead control were aspirated until empty and 50  
14 microliters of 70% ethanol was added. The plate was then  
15 emptied by inverting and blotted dry. Room temperature PBS  
16 was dispensed into each well from a multichannel squeeze  
17 bottle, tapped three times, emptied by inversion and then  
18 blotted dry. 50 microliters of the fluorescent  
19 ~~Live/Dead~~LIVE/DEAD dye diluted in PBS was added to each well  
20 and incubated at 37°C in a 5% CO<sub>2</sub> incubator for one hour. The  
21 plates were read in a Perkin-Elmer HTS7000 fluorescence plate  
22 reader and the data was analyzed in Microsoft Excel.

23 Four rounds of screening were conducted to produce  
24 single clone hybridoma cultures. For two rounds of screening  
25 the hybridoma supernatants were tested only against the

1 cancer cells. In the last round of screening the supernatant  
2 was tested against a number of non-cancer cells as well as  
3 the target cells indicated in table 1. The antibodies were  
4 isotyped using a commercial isotyping kit.

5 A number of monoclonal antibodies were produced in  
6 accordance with the method of the present invention. These  
7 antibodies, whose characteristics are summarized in Table 1,  
8 are identified as 3BD-3, 3BD-6, 3BD-8, 3BD-9, 3BD-15, 3BD-25,  
9 3BD-26 and 3BD-27. Each of the designated antibodies is  
10 produced by a hybridoma cell line deposited with the American  
11 Type Culture Collection at 10801 University Boulevard,  
12 Manassas, Va. having an ATCC Accession Number as follows:

13	<u>Antibody</u>	<u>ATCC Accession Number</u>
14	3BD-3	
15	<del>3BD-6</del>	
16	<del>3BD-8</del>	
17	<del>3BD-9</del>	
18	<del>3BD-15</del>	
19	<del>3BD-25</del>	
20	<del>3BD-26</del>	
21	<del>3BD-27</del>	not deposited
22	3BD-6	not deposited
23	3BD-8	PTA-2696
24	3BD-9	not deposited

1 3BD-15 not deposited  
2 3BD-25 not deposited  
3 3BD-26 PTA-2695  
4 3BD-27 PTA-2698

5 These antibodies are considered monoclonal after four rounds  
6 of limiting dilution cloning. The anti-melanoma antibodies  
7 did not produce significant cancer cell killing. The panel of  
8 anti-breast cancer antibodies killed 32-87% of the target  
9 cells and <1-3% of the control cells. The predominant isotype  
10 was IgG1 even though it was expected that the majority of  
11 anti-tumor antibodies would be directed against carbohydrate  
12 antigens, and thus, be of the IgM type. There is a high  
13 therapeutic index since most antibodies spare the control  
14 cells from cell death.

16 Table 1. Anti-Breast Cancer Antibodies

Clones	% Cell Death			
	Target for Anti-Breast Cancer Antibodies (HEY & A2058)	Normal Fibroblast Cells (CCD-12CoN)	Fibrocystic Breast Cells (MCF-12A)	Isotype
3BD-3	74.9%	3.7%	<1%	$\gamma$ 1, $\lambda$
3BD-6	68.5%	5.6%	<1%	$\gamma$ 1, $\lambda$
3BD-8	81.9%	4.5%	2.6%	$\gamma$ 1, $\kappa$
3BD-9	77.2%	7.9%	<1%	$\gamma$ 1, $\lambda$
3BD-15	87.1%	<1 %	<1%	$\gamma$ 1, $\lambda$

3BD-26	54.8%	3.3%	<1%	μ,κ
3BD-25	32.4%	3.6%	<1 %	γ1.κ
3BD-27	60.1%	8.3%	1.3%	γ1. κ

## ~~Example~~EXAMPLE 2

In this example customized anti-cancer antibodies are produced by first obtaining samples of the patient's tumor. Usually this is from a biopsy specimen from a solid tumor or a blood sample from hematogenous tumors. The samples are prepared into single cell suspensions and fixed for injection into mice. After the completion of the immunization schedule the hybridomas are produced from the splenocytes. The hybridomas are screened against a variety of cancer cell lines and normal cells in standard cytotoxicity assays. Those hybridomas that are reactive against cancer cell lines but are not reactive against normal non-transformed cells are selected for further propagation. Clones that were considered positive were ones that selectively killed the cancer cells but did not kill the non-transformed cells. The antibodies are characterized for a large number of biochemical parameters and then humanized for therapeutic use.

The melanoma tumor cells isolated and cell lines were cultured as described in Example 1. Balb/c mice were immunized according to the following schedule: 200,000 cells s.c. and i.p. on day 0, then 200,000 cells were injected i.p.

1 on day 21, then 1,000,000 cells were injected on day 49, then  
2 1,250,000 cells in Freund's Complete Adjuvant were injected  
3 i.p. on day 107, and then 200,000 cells were injected on day  
4 120 i.p. and then the mice were sacrificed on day 123. The  
5 spleens were harvested and the splenocytes were divided into  
6 two aliquots for fusion with Sp2/0 (1LN) or NS-1 (2LN)  
7 myeloma partners using the methods outlined in example 1.

8 The screening was carried out 11 days after the fusion  
9 against A2058 melanoma cells and CCD-12CoN fibroblasts. Each  
10 pair of plates were washed with 100 microliters of room  
11 temperature PBS and then aspirated to near dryness. Then 50  
12 microliters of hybridoma supernatant was added to the same  
13 wells on each of the two plates. The spent Sp2/0 supernatant  
14 was added to the control wells at the same volume and the  
15 plates were incubated for around 18 hours at 37 degrees  
16 Celsius at a 8%CO<sub>2</sub>, 98% relative humidity incubator. Then  
17 each pair of plates were removed and in the positive control  
18 wells 50 microliters of 70% ethanol was substituted for the  
19 media for 4 seconds. The plates were then inverted and washed  
20 with room temperature PBS once and dried. Then 50uL of  
21 fluorescent ~~live/dead~~LIVE/DEAD dye diluted in PBS (Molecular  
22 Probes ~~Live/Dead~~LIVE/DEAD Kit) was added for one hour and  
23 incubated at 37 degrees Celsius. The plates were then read in  
24 a Perkin-Elmer fluorescent plate reader and the data analyzed  
25 using Microsoft Excel. The wells that were considered

positive were subcloned and the same screening process was repeated 13 days later and then 33 days later. The results of the last screening is outlined in Table 2 below. A number of monoclonal antibodies were produced in accordance with the method of the present invention. These antibodies, whose characteristics are summarized in Table 2, are identified as 1LN-1, 1LN-8, 1LN-12, 1LN-14, 2LN-21, 2LN-28, 2LN-29, 2LN-31, 2LN-33, 2LN-34 and 2LN-35. Each of the designated antibodies is produced by a hybridoma cell line deposited with the American Type Culture Collection at 10801 University Boulevard, Manassas, Va. having an ATCC Accession Number as follows:

<u>Antibody</u>	<u>ATCC Accession Number</u>
1LN-1	
<del>1LN-8</del>	
<del>1LN-12</del>	
<del>1LN-14</del>	
<del>2LN-21</del>	
<del>2LN-28</del>	
<del>2LN-29</del>	
<del>2LN-31</del>	
<del>2LN-33</del>	

1	<del>2LN-34</del>	
2	<del>2LN-35</del>	not deposited
3	1LN-8	PTA-2693
4	1LN-12	not deposited
5	1LN-14	not deposited
6	2LN-21	not deposited
7	2LN-28	not deposited
8	2LN-29	not deposited
9	2LN-31	not deposited
10	2LN-33	not deposited
11	2LN-34	not deposited
12	2LN-35	not deposited

13 Table 2, Anti-Melanoma Antibodies

Clones	% Cell Death	
	Target for Anti-Melanoma Antibodies (A2058)	Normal Fibroblast Cells (CCD-1 2CoN)
1LN-1	59.4%	<1 %
1LN-8	11.0%	5.0%
1LN-12	55.2%	1.4%
1LN-14	51.4%	<1%
2LN-21	72.0%	15.9%
2LN-28	66.6%	12.4%
2LN-29	78.2%	6.1%
2LN-31	100%	7.8%
2LN-33	94.2%	<1%
2LN-34	56.6%	11.2%



2LN-35	66.5%	6.6%
--------	-------	------

The table illustrates that clones from both the Sp2/0 and NS-1 fusions were able to produce antibodies that had a greater than 50% killing rate for cancerous cells and at the same time some of the clones were able to produce less than one percent killing of normal control fibroblasts.

### ~~Example~~EXAMPLE 3

In this example antibodies were produced to several different breast tumor samples following the method of Example 2 in order to demonstrate the generality of producing customized antibodies. Biopsy specimens of breast tumors were obtained and stored at -70°C until used as noted in Example 1. Single cell suspensions were prepared for each specimen and fixed with -30°C, 70% ethanol, washed with PBS and reconstituted to an appropriate volume for injection. Female, 7-8 week old, A strain, H-2<sup>d</sup> haplotype Balb/c mice (Charles River Canada, St. Constant, QC, Can), were immunized with  $2.5 \times 10^5$ - $1 \times 10^6$  cells and boosted every third week until a final pre-fusion boost was performed three days prior to the splenectomy. The hybridomas were prepared by fusing the isolated splenocytes with Sp2/0 myeloma partners. The supernatants from the fusions were tested for subcloning of the hybridomas.

1           Hs574.T breast ductal carcinoma cells, A2058 melanoma  
2   cells, NCI-H460 human lung large cell carcinoma, NCI-H661  
3   human lung large cell carcinoma, CCD-112CoN human colon  
4   fibroblasts, CCD-27sk human skin fibroblasts, MCF-12A human  
5   mammary epithelial cells, Hs574.mg human breast cells and  
6   other cell lines, were obtained from ATCC and cultured  
7   according to enclosed instructions. Both cancer and non-  
8   cancer cells were plated three to four days prior to  
9   screening.

10          The hybridomas were cultured for ten to twelve days  
11   after fusion and observed under the microscope. When 20 to  
12   25% of the wells were over 80% confluent, the hybridoma  
13   supernatants were screened in a cytotoxicity assay. The  
14   hybridoma supernatants were divided into two 75 microliter  
15   portions; one portion was added to a target cancer cell plate  
16   and another to a non-cancer cell plate. Prior to transfer of  
17   hybridoma supernatants, the cell plates were washed three  
18   times with 100 microliters of PBS. The supernatant from the  
19   anti-breast cancer hybridomas were transferred to the Hs574.T  
20   and the Hs574.mg cells, whereas the supernatant from the  
21   anti-lung cancer hybridoma were transferred to the NCI-H460  
22   and CCD-27SK cells. The cancer cells were incubated with the  
23   hybridoma supernatants for 18 hours at 37 degrees Celsius in  
24   an 8 percent CO<sub>2</sub> incubator.

1       The ~~Live/Dead~~LIVE/DEAD cytotoxicity assay was obtained  
2       from Molecular Probes (Eugene,OR). The assays were performed  
3       according to the manufacturer's instructions with the changes  
4       outlined below. The plates with the cells were washed once  
5       with 100 microliters of PBS at 37°C. 75 to 100 microliters  
6       of supernatant from the hybridoma microtitre plates were  
7       transferred to the cell plates and incubated in a 8% CO<sub>2</sub>  
8       incubator for 18-24 hours. Then, the wells that served as the  
9       dead control cells were aspirated until empty and 50  
10       microliters of 70% ethanol was added. The plate was then  
11       emptied by inverting and blotted dry. Room temperature PBS  
12       was dispensed into each well from a multichannel squeeze  
13       bottle, tapped three times, emptied by inversion and then  
14       blotted dry. 50 microliters of the fluorescent  
15       ~~Live/Dead~~LIVE/DEAD dye diluted in PBS was added to each well  
16       and incubated at 37°C in a 5% CO<sub>2</sub> incubator for one hour. The  
17       plates were read in a Perkin-Elmer HTS7000 fluorescence plate  
18       reader and the data was analyzed in Microsoft Excel  
19       (Microsoft, Redmond, WA).

20       Four rounds of screening were conducted to produce  
21       single clone hybridoma cultures. For two rounds of screening  
22       the hybridoma supernatants were tested only against the  
23       cancer cells. In the last round of screening the supernatant  
24       was tested against a number of non-cancer cells as well as  
25       the target cells indicated in Table 3. The antibodies were

1 isotyped using a commercial isotyping kit (Roche,  
2 Indianapolis, IN).

3 A number of monoclonal antibodies were produced in  
4 accordance with the method of the present invention. These  
5 antibodies, whose characteristics are summarized in Table 3,  
6 are identified as 4BD-1, 4BD-3, 4BD-6, 4BD-9, 4BD-13, 4BD-18,  
7 4BD-20, 4BD-25, 4BD-37, 4BD-32, 4BD-26, 4BD-27, 4BD-28, 4BD-  
8 50, 6BD-1, 6BD-3, 6BD-5, 6BD-11, 6BD-25, 7BD-7, 7BD-12-1,  
9 7BD-12-2, 7BD-13, 7BD-14, 7BD-19, 7BD-21, 7BD-24, 7BD-29,  
10 7BD-30, 7BD-31, 7BDI-17, 7BDI-58, 7BDI-60 and 7BDI-62. Each  
11 of the designated antibodies is produced by a hybridoma cell  
12 line deposited with the American Type Culture Collection at  
13 10801 University Boulevard, Manassas, Va. having an ATCC  
14 Accession Number as follows:

15	<u>Antibody</u>	<u>ATCC Accession Number</u>
16	4BD-1	
17	<del>4BD-3</del>	
18	<del>4BD-6</del>	
19	<del>4BD-9</del>	
20	<del>4BD-13</del>	
21	<del>4BD-18</del>	
22	<del>4BD-20</del>	
23	<del>4BD-25</del>	
24	<del>4BD-37</del>	
25	<del>4BD-32</del>	

1	<del>4BD-26</del>	
2	<del>4BD-27</del>	
3	<del>4BD-28</del>	
4	<del>4BD-50</del>	
5	<del>6BD-1</del>	
6	<del>6BD-3</del>	
7	<del>6BD-5</del>	
8	<del>6BD-11</del>	
9	<del>6BD-25</del>	
10	<del>7BD-7</del>	
11	<del>7BD-12-1</del>	
12	<del>7BD-12-2</del>	
13	<del>7BD-13</del>	
14	<del>7BD-14</del>	
15	<del>7BD-19</del>	
16	<del>7BD-21</del>	
17	<del>7BD-24</del>	
18	<del>7BD-29</del>	
19	<del>7BD-30</del>	
20	<del>7BD-31</del>	
21	<del>7BDI-17</del>	
22	<del>7BDI-58</del>	
23	<del>7BDI-60</del>	
24	<del>7BDI-62</del>	not deposited
25	4BD-3	not deposited
26	4BD-6	not deposited

1	4BD-9	not deposited
2	4BD-13	not deposited
3	4BD-18	not deposited
4	4BD-20	not deposited
5	4BD-25	not deposited
6	4BD-37	not deposited
7	4BD-32	not deposited
8	4BD-26	not deposited
9	4BD-27	not deposited
10	4BD-28	not deposited
11	4BD-50	not deposited
12	6BD-1	not deposited
13	6BD-3	not deposited
14	6BD-5	not deposited
15	6BD-11	not deposited
16	6BD-25	not deposited
17	7BD-7	not deposited
18	7BD-12-1	not deposited
19	7BD-12-2	not deposited
20	7BD-13	not deposited
21	7BD-14	PTA-2697
22	7BD-19	not deposited
23	7BD-21	not deposited
24	7BD-24	not deposited
25	7BD-29	not deposited
26	7BD-30	not deposited

1	7BD-31	not deposited
2	7BDI-17	not deposited
3	7BDI-58	not deposited
4	7BDI-60	PTA-4623
5	7BDI-62	not deposited

6        These antibodies are considered monoclonal after four  
7 rounds of limiting dilution cloning. The panel of anti-breast  
8 cancer antibodies killed 15-79% of the target cells and <1-  
9 31% of the control cells. The majority of anti-tumor  
10 antibodies were IgM type, suggesting they could be directed  
11 against carbohydrate antigens on the surface of tumor cells.  
12 There is a high therapeutic index since most of the  
13 antibodies do not cause the normal cells to undergo cell  
14 death.

15        These monoclonal antibodies are characterized for a number  
16 of immunological and biochemical parameters. A cell based  
17 enzyme linked immunosorbent assay (ELISA) was established for  
18 measuring the binding of the antibodies derived of each  
19 clones to different cell lines. Cells were seeded and grown  
20 on 96-well tissue culture plates. The plates were washed with  
21 100 microliters of PBS. 100 microliters of cold 4 percent  
22 paraformaldehyde in PBS were added to each well for ten  
23 minutes and then aspirated. The plates were washed with PBS  
24 using a multichannel squeeze bottle . The wells were emptied  
25 and 100 microliters of blocking buffer (1 percent

1 hydrocasein, 0.1 percent geletin in 50mM Tris-HCl buffer, pH  
2 9.3) was added to each well for one hour at room temperature.  
3 The plates were washed three times with a buffer (0.05  
4 percent Tween 20 in 10 mM PBS) at room temperature and then  
5 stored at -30 degrees Celsius with 100 microliters of the  
6 buffer. Prior to use the plates were thawed and the buffer  
7 was aspirated from each well. 75 microliters of hybridoma  
8 supernatant were added to each well and incubated for 60  
9 minutes at room temperature. After the plates were washed  
10 with PBS using a multichannel squeeze bottle, 50 microliters  
11 of a combination of peroxidase conjugated goat anti-mouse IgG  
12 and peroxidase conjugated donkey anti-mouse IgM (Jackson  
13 ImmunoResearch Lab, Inc., West Grove, PA.) was added and  
14 incubated for 30 minutes at room temperature. After the last  
15 wash, 50 microliters of orthophenylene diamine (OPD) (Sigma,  
16 St. Louis, MO) was added to each well and the optical density  
17 was read at 492 nm on the HTS7000 plate reader after adding  
18 equal volume of 1 N sulfuric acid. Different clones show  
19 different profiles in binding to different cells (Table 3).  
20 This indicates that they may target different cell surface  
21 antigen and further suggests the variable distribution of  
22 these antigen on the surface of cancer cells. Those binding  
23 only to cancer cells but not to normal cells could identify  
24 certain tumor-associated antigen.

25



1 Table 3. Anti-Breast Cancer Antibodies

Clones	Isotype	% Cell Death		Binding to cell lines				
		Hs574.T	Hs574.mg	Hs574.T	Hs574.mg	NCI-H460	CCD-27sk	A2058
6BD-1	μ, κ	38.2	5	0.8	0.5	0.6	0.3	ND*
6BD-3	μ, κ	79	12	0.35	0.25	0.24	0.14	ND
6BD-5	μ, κ	57.3	8	1.0	0.3	0.14	0.25	ND
6BD-11	μ, κ	52.3	11	0.15	0.1	0.17	0.1	ND
6BD-25	μ, κ	33.3	2	0.15	0.1	0.2	0.1	ND
4BD-26	μ, κ	27	1.8	0.5	ND	ND	<0.1	ND
4BD-27	μ, κ	19.6	<1	0.9	ND	ND	0.5	ND
4BD-28	μ, κ	26.4	<1	0.8	ND	ND	<0.1	ND
4BD-32	μ, κ	41.7	4	0.8	ND	ND	<0.1	ND
4BD-50	μ, κ	20	<1	0.8	ND	ND	<0.1	ND
4BD-1	μ, κ	23	31	0.6	ND	ND	<0.1	ND
4BD-3	μ, κ	29.7	8.2	1.7	ND	ND	0.1	ND
4BD-6	μ, κ	17	<1	0.9	ND	ND	<0.1	ND
4BD-9	μ, κ	15	<1	0.6	ND	ND	<0.1	ND
4BD-13	μ, κ	31	<1	1.2	ND	ND	<0.1	ND
4BD-18	μ, κ	23.3	2.4	0.7	ND	ND	0.12	ND
4BD-20	μ, κ	45	<1	0.95	ND	ND	<0.1	ND
4BD-25	μ, κ	26	14.16	1.8	ND	ND	0.1	ND
4BD-37	μ, κ	30	<1	0.8	ND	ND	<0.1	ND
7BD-7	μ, κ	24	3	0.8	0.3	1.4	0.26	ND
7BD-12-1	μ, κ	22	6	0.36	0.16	0.43	0.1	ND
7BD-12-2	μ, κ	31	2	0.2	0.2	0.2	0.2	0.2
7BD-13	μ, κ	29	12	0.1	0.15	0.2	0.1	0.2
7BD-14	μ, κ	32	13	0.4	0.4	0.6	0.3	0.5
7BD-19	μ, κ	20	4	1.3	0.4	0.43	0.2	ND
7BD-21	μ, κ	21	13	0.4	0.5	0.25	0.3	ND
7BD-24	μ, κ	32	15	0.3	0.1	0.14	0.15	ND
7BD-29	μ, κ	15	16	0.3	0.24	0.14	0.16	ND

7BD-30	$\mu$ , K	23	13	0.34	0.24	0.2	0.16	ND
7BD-31	$\mu$ , K	28	10	0.3	0.4	0.4	0.3	0.4
7BDI-17	$\mu$ , K	23	<1	0.75	ND	ND	ND	ND
7BDI-58	$\gamma$ 1, K	17.5	<1	0.77	ND	ND	ND	ND
7BDI-60	$\gamma$ 1, K	15	<1	0.73	ND	ND	ND	ND
7BDI-62		15	5	0.55	ND	ND	ND	ND

\*ND: not done.

#### ~~Example~~EXAMPLE 4

In this example customized anti-cancer antibodies are produced to a lung cancer sample by first obtaining samples of the patient's tumor preparing single cell suspensions which are then fixed for injection into mice as noted in Example 1. After the completion of the immunization schedule the hybridomas are produced from the splenocytes. The hybridomas are screened against a variety of cancer cell lines and normal cells in standard cytotoxicity assays. Those hybridomas that are reactive against cancer cell lines but are not reactive against normal non-transformed cells are selected for further propagation. Clones that were considered positive were ones that selectively killed the cancer cells but did not kill the non-transformed cells.

The lung cancer cells were isolated and cell lines were cultured as described in Example 1. Female, 7-8 week old, A strain, H-2<sup>d</sup> haplotype Balb/c mice (Charles River Canada, St. Constant, QC, Can), were immunized with human

1 lung cancer cells. The lung cancer cell suspensions were  
2 emulsified in an equal volume of Freund's complete adjuvant  
3 (FCA) for the first immunization and then in Freund's  
4 incomplete adjuvant (FIA) for subsequent immunizations at 0,  
5 21, 45 days.  $5 \times 10^5$  cells were used to immunize each mouse  
6 either through a subcutaneous or intra-peritoneal route.  
7 Immunized mice were sacrificed 3-4 days after the final  
8 immunization with human lung cancer cells at 148 days, given  
9 intra-peritoneally, in PBS at pH 7.4. The spleens were  
10 harvested and the splenocytes were divided into two aliquots  
11 for fusion with Sp2/0 myeloma partners using the methods  
12 outlined in Example 1.

13 The screening was carried out 10 days after the fusion  
14 against NCI-H460 and/or NCI-H661 cells and CCD-27SK  
15 fibroblasts. Each pair of plates were washed with 100  
16 microliters of room temperature PBS and then aspirated to  
17 near dryness. Then 75 microliters of hybridoma supernatant  
18 was added per well on each of the two plates. The spent Sp2/0  
19 supernatant was added to the control wells at the same volume  
20 and the plates were incubated for around 18 hours at 37  
21 degrees Celsius at a 8%CO<sub>2</sub>, 98% relative humidity incubator.  
22 Then each pair of plates was removed and in the positive  
23 control wells 50 microliters of 70% ethanol was substituted  
24 for the media for 4 seconds. The plates were then inverted  
25 and washed with room temperature PBS once and dried. Then 50

1 microliters of fluorescent live/dead dye diluted in PBS  
2 (Molecular Probes ~~Live/Dead~~LIVE/DEAD Kit) was added for one  
3 hour and incubated at 37 degrees Celsius. The plates were  
4 then read in a Perkin-Elmer fluorescent plate reader and the  
5 data analyzed using Microsoft Excel. The wells that were  
6 considered positive were subcloned and the same screening  
7 process was repeated 6 days later and then 13 days later. The  
8 result of the last screening is outlined in Table 4 below.  
9 Antibodies were characterized for binding to different cell  
10 lines with a cellular ELISA according to the methods of  
11 Example 3. A number of monoclonal antibodies were produced in  
12 accordance with the method of the present invention. These  
13 antibodies, whose characteristics are summarized in Table 4,  
14 are identified as 5LAC2, 5LAC4, 5LAC20, and 5LAC23. Each of  
15 the designated antibodies is produced by a hybridoma cell  
16 line deposited with the American Type Culture Collection at  
17 10801 University Boulevard, Manassas, Va. having an ATCC  
18 Accession Number as follows:

<u>Antibody</u>	<u>ATCC Accession Number</u>
5LAC2	
<del>5LAC4</del>	
<del>5LAC20</del>	
<del>5LAC23</del>	not deposited
5LAC4	not deposited

1 5LAC20 PTA-2694

2 5LAC23 not deposited

3

4 Table 4. Anti-Lung Cancer Antibodies

Clones	Isotype	% Cell Death					Binding to cell lines				
		Hs574.T	NCI-H460	NCI-H661	A2058	CCD-27sk	Hs574.T	Hs574.mg	NCI- H460	CCD-27sk	A2058
5LAC2	μ, κ	30	7	45.3	23	<1	0.2	0.2	0.26	0.2	0.2
5LAC4	μ, κ	21	11	20.5	23	3	0.7	0.9	1.7	0.8	0.9
5LAC20	μ, κ	23	7	66.4	24	3	0.5	0.2	0.6	0.2	0.2
		23	8	57.6	25	5	0.6	0.6	0.6	0.6	0.6

15 \*ND: not done

16 The table illustrates that clones were able to produce  
17 antibodies that had a greater than 7-67% killing rate for  
18 cancerous cells and at the same time some of the clones were  
19 able to produce less than five percent killing of normal  
20 control fibroblasts.

21

22 ~~Example~~EXAMPLE 5

23 In this example customized anti-cancer antibodies are  
24 produced to a patient's lung cancer cells, but cultured cells  
25 were used in the antibody development process to demonstrate  
26 the generality of the immunization process. The samples were  
27 prepared into single cell suspensions and fixed for injection  
28 into mice as noted in Example 1. After the completion of

1 three rounds of immunization with cells derived directly from  
2 a patient's lung cancer, the mice were immunized twice with a  
3 human lung large cell carcinoma cell line (NCI-H460).

4 Hybridomas were produced from splenocytes and the  
5 supernatants were screened against a variety of cancer cell  
6 lines and normal cells in standard cytotoxicity assays. Those  
7 hybridomas that were reactive against cancer cell lines but  
8 were not reactive against normal non-transformed cells were  
9 selected for further propagation. Clones that were considered  
10 positive were ones that selectively killed the cancer cells  
11 but did not kill the non-transformed cells. The antibodies  
12 are characterized for a large number of biochemical  
13 parameters and then humanized for therapeutic use.

14 The lung tumor cells isolated and cell lines were  
15 cultured as described in Example 1. Balb/c mice, A strain  
16 with H-2<sup>d</sup> haplotype from Charles River Canada, St. Constant,  
17 Quebec, Canada, female, 7-8 week old, were immunized with the  
18 human lung cancer cells emulsified in an equal volume of  
19 either Freund's complete adjuvant (FCA) for the first  
20 immunization and then in Freund's incomplete adjuvant (FIA)  
21 for subsequent immunizations at 0, 21, 45 days with  $5 \times 10^5$   
22 cells. The mice were immunized with fixed NCI H460 cells,  
23 which were prepared from NCI H460 cells grown in T-75 cell  
24 culture flask by scraping mono-layer cells into cell  
25 suspensions at 105, 150 and 170 days. Immunized mice were

1 sacrificed 3-4 days after the final immunization with NCI  
2 H460 cells, given intra-peritoneally, in phosphate buffered  
3 saline buffer (PBS), pH 7.4. The spleens were harvested and  
4 the splenocytes were divided into two aliquots for fusion  
5 with Sp2/0 myeloma partners using the methods outlined in  
6 Example 1.

7 The screening was carried out 10 days after the fusion  
8 against NCI H460 cells and CCD-27SK fibroblasts as described  
9 in Example 4. Antibodies were characterized for binding to  
10 different cell lines with a cellular ELISA according to the  
11 methods of Example 3.

12 ———The wells that were considered positive were  
13 subcloned and the same screening process was repeated 9 days  
14 and 18 days later. The results are outlined in Table 5 below.  
15 A number of monoclonal antibodies were produced in accordance  
16 with the method of the present invention. These antibodies,  
17 whose characteristics are summarized in Table 5, are  
18 identified as H460-1, H460-4, H460-5, H460-10, H460-14, H460-  
19 16-1, H460-16-2, H460-23 and H460-27. Each of the designated  
20 antibodies is produced by a hybridoma cell line deposited  
21 with the American Type Culture Collection at 10801 University  
22 Boulevard, Manassas, Va. having an ATCC Accession Number as  
23 follows:

24	<u>Antibody</u>	<u>ATCC Accession Number</u>
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25	H460-1	
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1	<del>H460-4</del>	
2	<del>H460-5</del>	
3	<del>H460-10</del>	
4	<del>H460-14</del>	
5	<del>H460-16-1</del>	
6	<del>H460-16-2</del>	
7	H460-23	
8	H460-27	not deposited
9	H460-4	not deposited
10	H460-5	not deposited
11	H460-10	not deposited
12	H460-14	not deposited
13	H460-16-1	not deposited
14	H460-16-2	PTA-4621
15	H460-23	PTA-2700
16	H460-27	PTA-2699

17 Table 5. Anti-Lung Cancer Antibodies

Clones	isotype	% Cell Death				Binding to cell lines				
		NCI-H460	Hs574.T	A2058	CCD-	Hs574.	Hs574.m	NCI-	CCD-	A2058
H460-1	$\gamma 1, \epsilon$	16	30	23	<1	1.0	0.6	0.5	0.7	ND
H460-4	$\gamma 1, \epsilon$	37	21	23	3	1.0	0.6	0.4	0.6	ND
H460-5	$\mu, \kappa$	22.5	23	24	3	1.0	0.3	0.3	0.2	ND

H460-10	$\mu, \kappa$	8	23	25	5	0.3	0.14	0.2	0.1	ND
H460-14	$\gamma 1, \epsilon$	17	ND	ND	4	1.1	0.6	0.4	0.54	ND
H460-16-1	$\gamma 1, \epsilon$	33	ND	ND	8	1.0	0.6	0.3	0.5	ND
H460-16-2	$\gamma 1, \epsilon$	22	ND	ND	3	1.0	0.6	0.3	0.7	ND
H460-22-1	$\gamma 1, \epsilon$	21	ND	ND	5	0.6	0.4	0.3	0.4	ND
H460-22-2	$\mu, \kappa$	23	ND	ND	3	0.4	0.1	0.1	0.1	ND
H460-23	$\mu, \kappa$	36	36	18	1	0.4	1.1	0.54	0.53	0.58
H460-27	$\mu, \kappa$	33	31	16	8	0.3	0.4	0.4	0.3	0.4

\*ND: not done

The table illustrates that clones were able to produce antibodies that had a greater than 15% killing rate for cancerous cells and at the same time some of the clones were able to produce less than eight percent killing of normal control fibroblasts.

The anti-cancer antibodies of the invention are useful for treating a patient with a cancerous disease when administered in admixture with a pharmaceutically acceptable adjuvant, for example normal saline, a lipid emulsion, albumen, phosphate buffered saline or the like and are administered in an amount effective to mediate treatment of said cancerous disease, for example with a range of about 1 microgram per milliliter to about 1 gram per milliliter.

The method for treating a patient suffering from a cancerous disease may further include the use of conjugated anti-cancer antibodies and would this include conjugating patient specific anti-cancer antibodies with a member

1 selected from the group consisting of toxins, enzymes,  
2 radioactive compounds, and hematogenous cells; and  
3 administering these conjugated antibodies to the patient;  
4 wherein said anti-cancer antibodies are administered in  
5 admixture with a pharmaceutically acceptable adjuvant, for  
6 example normal saline, a lipid emulsion, albumen, phosphate  
7 buffered saline or the like and are administered in an amount  
8 effective to mediate treatment of said cancerous disease, for  
9 example with a range of about 1 microgram per mil to about 1  
10 gram per mil. In a particular embodiment, the anti-cancer  
11 antibodies useful in either of the above outlined methods may  
12 be a humanized antibody.

13  
14 The anti-cancer antibodies of the invention are useful  
15 for treating a patient with a cancerous disease when  
16 administered in admixture with a pharmaceutically acceptable  
17 adjuvant, for example normal saline, a lipid emulsion,  
18 albumen, phosphate buffered saline or the like and are  
19 administered in an amount effective to mediate treatment of  
20 said cancerous disease, for example with a range of about 1  
21 microgram per mil to about 1 gram per mil.

22 The method for treating a patient suffering from a  
23 cancerous disease may further include the use of conjugated  
24 anti-cancer antibodies and would this include conjugating  
25 patient specific anti-cancer antibodies with a member

1 selected from the group consisting of toxins, enzymes,  
2 radioactive compounds, and hematogenous cells; and  
3 administering these conjugated antibodies to the patient;  
4 wherein said anti-cancer antibodies are administered in  
5 admixture with a pharmaceutically acceptable adjuvant, for  
6 example normal saline, a lipid emulsion, albumen, phosphate  
7 buffered saline or the like and are administered in an amount  
8 effective to mediate treatment of said cancerous disease, for  
9 example with a range of about 1 microgram per mil to about 1  
10 gram per mil. In a particular embodiment, the anti-cancer  
11 antibodies useful in either of the above outlined methods may  
12 be a humanized antibody.

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CLAIMS

What is claimed is:

Claim 1. A method for treating a patient suffering from a cancerous disease comprising:

administering to said patient anti-cancer antibodies or fragments thereof produced in accordance with a method for the production of individually customized anti-cancer antibodies which are useful in treating a cancerous disease, said antibodies including a subset of antibodies or fragments thereof characterized as being cytotoxic against cells of a cancerous tissue, said subset being essentially benign to non-cancerous cells;

wherein one or more antibodies or fragments thereof selected from said subset are placed in admixture with a pharmaceutically acceptable adjuvant and are administered in

1 an amount effective to mediate treatment of said cancerous  
2 disease;

3 said one or more antibodies or fragments thereof being  
4 selected from the group consisting of a 1LN-8, 4BD-1, a 4BD-  
5 3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-20, a 4BD-25,  
6 a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a 4BD-37, a 4BD-50, a  
7 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a 6BD-25, a 7BD-7, a 7BD-  
8 12-1, a 7BD-12-2, a 7BD-13, a 7BD-14, a 7BD-19, a 7BD-21, a  
9 7BD-24, a 7BD-29, a 7BD-30, a 7BD-31, a 7BDI-17, a 7BDI-58, a  
10 7BDI-60, a 7BDI-62, a 5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a  
11 H460-1, a H460-4, a H460-5, a H460-10, a H460-14, a H460-16-  
12 1, a H460-16-2, a H460-23 and a H460-27 monoclonal antibody  
13 or combinations thereof.

14  
15 Claim 2. The method for treating a patient suffering  
16 from a cancerous disease in accordance with claim 1, wherein  
17 said one or more antibodies or fragments thereof selected  
18 from said subset are humanized.

19  
20 Claim 3. The method for treating a patient suffering  
21 from a cancerous disease in accordance with claim 1  
22 comprising:

23 conjugating said subset of antibodies or fragments  
24 thereof with a member selected from the group consisting of  
25 toxins, enzymes, radioactive compounds, and hematogenous  
26 cells; and

1 administering conjugated antibodies or fragments thereof  
2 to said patient;

3 wherein said conjugated antibodies are placed in  
4 admixture with a pharmaceutically acceptable adjuvant and are  
5 administered in an amount effective to mediate treatment of  
6 said cancerous disease.

7  
8 Claim 4. The method of claim 3, wherein said one or  
9 more antibodies or fragments thereof selected from said  
10 subset are humanized.

11  
12 Claim 5. The method for treating a patient suffering  
13 from a cancerous disease in accordance with claim 1 wherein:  
14 the cytotoxicity of said antibodies or fragments thereof  
15 is mediated through antibody dependent cellular toxicity.

16  
17 Claim 6. The method for treating a patient suffering  
18 from a cancerous disease in accordance with claim 1 wherein:  
19 the cytotoxicity of said antibodies or fragments thereof  
20 is mediated through complement dependent cellular toxicity.

21  
22 Claim 7. The method for treating a patient suffering  
23 from a cancerous disease in accordance with claim 1 wherein:  
24 the cytotoxicity of said antibodies or fragments thereof  
25 is mediated through catalyzing of the hydrolysis of cellular  
26 chemical bonds.

1        Claim 8. The method for treating a patient suffering  
2        from a cancerous disease in accordance with claim 1 wherein:  
3        the cytotoxicity of said antibodies or fragments thereof  
4        is mediated through producing an immune response against  
5        putative cancer antigens residing on tumor cells.

6  
7        Claim 9. The method for treating a patient suffering  
8        from a cancerous disease in accordance with claim 1 wherein:  
9        the cytotoxicity of said antibodies or fragments thereof  
10       is mediated through targeting of cell membrane proteins to  
11       interfere with their function.

12  
13       Claim 10. The method for treating a patient suffering  
14       from a cancerous disease in accordance with claim 1 wherein:  
15       the cytotoxicity of said antibodies or fragments thereof  
16       is mediated through production of a conformational change in  
17       a cellular protein effective to produce a signal to initiate  
18       cell-killing.

19  
20       Claim 11. The method for treating a patient suffering  
21       from a cancerous disease in accordance with claim 1 wherein:  
22       said method of production utilizes a tissue sample  
23       containing cancerous and non-cancerous cells obtained from a  
24       particular individual.



1           Claim 12. A method for treating a patient suffering from  
2 a cancerous disease comprising: ✓

3           administering to said patient anti-cancer antibodies or  
4 fragments thereof produced in accordance with a method for  
5 the production of individually customized anti-cancer  
6 antibodies which are useful in treating a cancerous disease,  
7 said antibodies including a subset of antibodies or fragments  
8 thereof characterized as being cytotoxic against cells of a  
9 cancerous tissue, said subset being essentially benign to  
10 non-cancerous cells;

11           wherein one or more antibodies or fragments thereof  
12 selected from said subset are placed in admixture with a  
13 pharmaceutically acceptable adjuvant and are administered in  
14 an amount effective to mediate treatment of said cancerous  
15 disease;

16           said one or more antibodies or fragments thereof  
17 produced by a hybridoma cell line having an ATCC Accession  
18 Number selected from the group consisting of (        ) or  
19 combinations thereof.  
20

21           Claim 13. The method for treating a patient suffering  
22 from a cancerous disease in accordance with claim 12, wherein  
23 said one or more antibodies or fragments thereof selected  
24 from said subset are humanized.  
25

1           Claim 14. The method for treating a patient suffering  
2   from a cancerous disease in accordance with claim 12  
3   comprising:

4           conjugating said subset of antibodies or fragments  
5   thereof with a member selected from the group consisting of  
6   toxins, enzymes, radioactive compounds, and hematogenous  
7   cells; and

8           administering conjugated antibodies or fragments thereof  
9   to said patient;

10          wherein said conjugated antibodies are placed in  
11   admixture with a pharmaceutically acceptable adjuvant and are  
12   administered in an amount effective to mediate treatment of  
13   said cancerous disease.

14  
15          Claim 15. The method of claim 14, wherein said one or  
16   more antibodies or fragments thereof selected from said  
17   subset are humanized.

18  
19          Claim 16. The method for treating a patient suffering  
20   from a cancerous disease in accordance with claim 12 wherein:  
21          the cytotoxicity of said antibodies or fragments thereof  
22   is mediated through antibody dependent cellular toxicity.

23  
24          Claim 17. The method for treating a patient suffering  
25   from a cancerous disease in accordance with claim 12 wherein:

1       the cytotoxicity of said antibodies or fragments thereof  
2       is mediated through complement dependent cellular toxicity.

3  
4       Claim 18. The method for treating a patient suffering  
5       from a cancerous disease in accordance with claim 12 wherein:  
6       the cytotoxicity of said antibodies or fragments thereof  
7       is mediated through catalyzing of the hydrolysis of cellular  
8       chemical bonds.

9  
10       Claim 19. The method for treating a patient suffering  
11       from a cancerous disease in accordance with claim 12 wherein:  
12       the cytotoxicity of said antibodies or fragments thereof  
13       is mediated through producing an immune response against  
14       putative cancer antigens residing on tumor cells.

15  
16       Claim 20. The method for treating a patient suffering  
17       from a cancerous disease in accordance with claim 12 wherein:  
18       the cytotoxicity of said antibodies or fragments thereof  
19       is mediated through targeting of cell membrane proteins to  
20       interfere with their function.

21  
22       Claim 21. The method for treating a patient suffering  
23       from a cancerous disease in accordance with claim 12 wherein:  
24       the cytotoxicity of said antibodies or fragments thereof  
25       is mediated through production of a conformational change in

1 a cellular protein effective to produce a signal to initiate  
2 cell-killing.

3  
4 Claim 22. The method for treating a patient suffering  
5 from a cancerous disease in accordance with claim 12 wherein:  
6 said method of production utilizes a tissue sample  
7 containing cancerous and non-cancerous cells obtained from a  
8 particular individual.

9  
10  
11  
12 Claim 23. Anti-cancer antibodies or fragments thereof  
13 selected from the group consisting of a 1LN-8, 4BD-1, a 4BD-  
14 3, a 4BD-6, a 4BD-9, a 4BD-13, a 4BD-18, a 4BD-20, a 4BD-25,  
15 a 4BD-26, a 4BD-27, a 4BD-28, a 4BD-32, a 4BD-37, a 4BD-50, a  
16 6BD-1, a 6BD-3, a 6BD-5, a 6BD-11, a 6BD-25, a 7BD-7, a 7BD-  
17 12-1, a 7BD-12-2, a 7BD-13, a 7BD-14, a 7BD-19, a 7BD-21, a  
18 7BD-24, a 7BD-29, a 7BD-30, a 7BD-31, a 7BDI-17, a 7BDI-58, a  
19 7BDI-60, a 7BDI-62, a 5LAC2, a 5LAC4, a 5LAC20, a 5LAC23, a  
20 H460-1, a H460-4, a H460-5, a H460-10, a H460-14, a H460-16-  
21 1, a H460-16-2, a H460-23 and a H460-27 monoclonal antibody  
22 or combinations thereof.

23  
24 Claim 24. Anti-cancer antibodies or fragments thereof  
25 produced by a hybridoma cell line having an ATCC Accession  
26 Number selected from the group

consisting of ( ).

**Abstract**

Claim 25. A binding assay to determine presence of cancerous

cells in a tissue sample selected from a tumor originating in colon, prostate, ovarian, lung, breast, or skin tissue comprising:

providing a tissue sample from a tumor originating in colon, prostate, ovarian, lung, breast, or skin tissue;

providing an isolated monoclonal antibody or antigen binding fragment thereof encoded by the clone deposited with the ATCC as Accession Number PTA-2700;

contacting said isolated monoclonal antibody or antigen binding fragment thereof with said tissue sample; and

determining binding of said isolated monoclonal antibody or antigen binding fragment thereof with said tissue sample;

1           whereby the presence of said cancerous cells in said  
2 tissue sample is indicated.

3  
4           Claim 26. A process of isolating or screening for  
5 cancerous cells in a tissue sample selected from a tumor  
6 originating in colon, prostate, ovarian, lung, breast, or  
7 skin tissue comprising:

8           providing a tissue sample from a tumor originating in  
9 colon, prostate, ovarian, lung, breast, or skin tissue;

10          providing an isolated monoclonal antibody or antigen  
11 binding fragment thereof encoded by the clone deposited with  
12 the ATCC as Accession Number PTA-2700;

13          contacting said isolated monoclonal antibody or antigen  
14 binding fragment thereof with said tissue sample; and

15          determining binding of said isolated monoclonal antibody  
16 or antigen binding fragment thereof with said tissue sample;

17          whereby said cancerous cells are isolated by said  
18 binding and their presence in said tissue sample is  
19 confirmed.

20                                   ABSTRACT

21           The present invention relates to a method for  
22 producing patient specific anti-cancer antibodies using a  
23 novel paradigm of screening. By segregating the anti-cancer  
24 antibodies using cancer cell cytotoxicity as an end point,  
25 the process makes possible the production of anti-cancer  
26 antibodies customized for the individual patient that can be

used for therapeutic and diagnostic purposes. The invention further relates to the process by which the antibodies are made and to their methods of use. The antibodies can be made specifically for one tumor derived from a particular patient and are selected on the basis of their cancer cell cytotoxicity and simultaneous lack of toxicity for non-cancerous cells. The antibodies can be used in aid of staging and diagnosis of a cancer, and can be used to treat tumor metastases. The anti-cancer antibodies can be conjugated to red blood cells obtained from that patient and re-infused for treatment of metastases based upon the recognition that metastatic cancers are usually well vascularized and the delivery of anti-cancer antibodies by red blood cells can have the effect of concentrating the antibodies at the site of the tumor.

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